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H. WAKEHAM

CONFIDENTIAL

WORK PLAN  
FOR  
"THE MECHANICAL AND BIOLOGICAL  
EVALUATION OF SMOKING MACHINES"

at the  
MASON RESEARCH INSTITUTE

November 12, 1969

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needed since injection of an antigen is required. One group of ten animals (half male and half female) will be injected with 0.25 ml of 50% thrice washed sheep erythrocytes in saline and smoking immediately initiated and continued for seven consecutive days. Mice will be exsanguinated on day eight and their sera will be tested for their anti-sheep erythrocyte response, hemagglutinin titer.

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between two to six weeks but it should be possible to study all four smoking machines and both phases of smoke during this same period of time.

Approximately 25% of the mice used in the LD<sub>50</sub> trials will be bled and tested for carboxyhemoglobin levels immediately after smoking and 60 and 120 minutes post-smoking. Carboxyhemoglobin levels will be correlated with carbon monoxide levels found in the smoking chamber during the LD<sub>50</sub> trials. Half the number of control bloods equally representing sham-smoked and cage controls will be used for carboxyhemoglobin determinations. Similar measurements will be carried out on survivors exposed to both whole smoke and gaseous phase in the LD<sub>50</sub> trials.

Phase 3: The establishment of an LD<sub>50</sub> will permit estimation of a maximum tolerated dose for acute daily exposures of mice in each smoking apparatus. Groups of ten animals (half male and half female) will receive exposure as determined to whole smoke or gaseous phase for 14, 28, or 56 days. Carbon monoxide levels in the smoking apparatuses will be measured once in the morning and once in the afternoon twice a week during the 56-day smoking study. An extra group will be smoked for 56 days but not sacrificed until day 70 in order to evaluate a two-week recovery interval. Gross observations will be made daily while each mouse will undergo weekly weighings. At the proper time sequence animals will be weighed, bled, sacrificed and specific tissues handled in three primary manners;

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Firstly, at necropsy a gross qualitative description of the appearances of lungs, liver and kidney will be recorded as well as the weights of adrenals, liver, lungs (wet and dry), kidney and heart. A limited number of lungs will undergo routine histology and histochemical estimation of cytochrome C-oxidase.

Secondly, lungs from six to eight mice will be analyzed for DNA, glycogen and lactate content while livers will be examined only for glycogen and lactate. Carboxyhemoglobin will be measured on individual blood samples and where possible, sera pools will be employed for the estimation of blood sugar, alkaline phosphatase, SGOT and SGPT.

surgeon's knot. The trachea is necked with scissors immediately below the cricoid cartilage to make an aperture just large enough to accept the polyethylene tubing. The surgeon's knot is tightened sufficiently to hold the tubing but not tied off. The xyphoid cartilage of the sternum is grasped with flat forceps and lifted while the ribs are excised laterally and removed. The heart is ligated at the atrial end. Measured amounts of O.C.T. or fixative are slowly expressed into the lungs, the tubing withdrawn and the suture immediately tied off. The trachea, lungs and heart are dissected from the thorax as one unit. The fixed lungs will be processed for paraffin sections which will be stained with H and E. The O.C.T. injected lungs will be solidified under fragments of dry ice and sectioned in the cryostat. Frozen sections will be incubated for the determination of cytochrome C-oxidase according to the procedure of Burstone.

#### 4. Biochemistry:

Representative samplings of phase two animals and half the number of a mixture of sham-smoked and cage-control mice will be bled suborbitally and the fresh blood examined spectrophotometrically before and after the addition of sodium hydrosulfite. The measurement of carboxy-hemoglobin will be performed immediately after a single exposure and at 60 and 120 minutes post-smoking and before necropsy. Since it is expected that 480 mice (see above) will be involved in the LD<sub>50</sub> trials, data will be collected on approximately 120 smoked mice and 60 control animals during phase two.

The continuous exposure studies (phase three) incorporate five blood parameters (carboxyhemoglobin, blood sugar, alkaline phosphatase, SGOT and SGPT) and two (liver glycogen and lactate) to four (lung dry weight, DNA, glycogen and lactate) tissue endpoints. After a final weighing,

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between puff and purge, purge duration and exposure time per puff. The evaluation of puff characteristics will relate to whole smoke.

## 2. Smoke composition:

As to the chemical profile of the smoke obtained from the reference cigarette two facets will be determined. Samples of smoke from randomized reference cigarettes will be introduced into a gas chromatograph fractionator to obtain a general profile of the major resolvable constituents. Such a profile will merely serve as a reference point as to the uniformity of the cigarettes and burning characteristics of each smoking apparatus. It is planned to obtain samples from the smoke chamber in the absence and presence of animals. In addition to the chromatographic profile the total reducing material in whole smoke and gaseous phase will be estimated by trapping such constituents in ethanol. Levels of blue-tetrazolium-formazan will be correlated with the weights of Cambridge filter pads when gaseous phase is studied. Sufficient tests will be carried out to be statistically valid.

Attempts will be made to monitor carbon monoxide in smoke chambers. Such measurements will be taken at those opportunities where CO levels can be correlated with the time study on carboxyhemoglobin. Estimations for each machine and for whole smoke and gaseous phase is anticipated. CO will be determined by gas chromatography.

## 3. Pathology:

Routine histology and histochemistry for cytochrome oxidase will be performed on a limited number of mice among the survivors of each group on the LD<sub>50</sub> trials for each smoking apparatus. It is estimated that 20 mice (half males and half females) will be necessary for each smoking apparatus at each air: smoke ratio to determine an LD<sub>50</sub> and maximum tolerated dose for one smoke phase. Therefore 20 mice x 3 air: smoke ratios x 2 smoke phases x 4 machines will add up to 480 mice (phase 2).

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As a special study to gather information on the reference cigarette and for partial monitoring of smoke components in the smoking machines, it is intended to measure a gas chromatographic profile ~~and the chemical reducing content~~ of whole smoke and gaseous-phase. The determination of carbon monoxide is also anticipated.

The parameters to measure puff characteristics will include puff volume, accuracy of volume delivery, puff duration, puff frequency, interval of time between puffs, interval between puff and purge, purge duration, the number of puffs per cigarette, and butt length.

The endpoints related to smoke exposure will incorporate determinations of the volume of the smoke chamber, exposure time per puff, dimensions and number of mouse containers, mobility of containers, animal loading time, loading stress, butt distance to animal, ability of mouse to block entry or exit of smoke, animal surface exposed and ability to visually observe the animal.

It is expected that phase one will be completed within two weeks of receipt and training of Institute personnel by the apparatus supplier. If all four machines arrive within the same week, it is planned to complete phase one for all machines within one month. This estimate is based on no break-down time. Serious failure of an apparatus will eliminate it from the study. If repair of a faulty apparatus is feasible, it will be performed by and at the expense of the supplier. Additional time will be required to fulfill phase one for the repaired smoking machine.

Phase 2: The reference cigarettes will be utilized for determining the acute LD<sub>50</sub> of whole smoke and gaseous phase (obtained by passing whole smoke through a Cambridge filter) for 25-35 g Swiss albino mice. Without prior knowledge as to the ease of regulation of the air: smoke ratio for a particular machine nor its animal capacity, only an estimate can be given for the time required to complete phase two. Such an estimate would fall

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Of this total approximately 10% will be used for routine histology and histochemistry. In the instance of the continuous smoking trials (phase 3) histology and histochemistry will require 5 mice x 4 sacrifice intervals (14, 28, 56 and 70 days) x 2 smoke phases x 4 smoking machines or a total of 160 mice. To this will be added 80 control mice, half sham-smoked and half cage controls. Therefore, for phase 2 and 3 approximately 300 mice will undergo routine histology and histochemical estimates of cytochrome C-oxidase.

Each animal utilized by pathology will have had weekly weighings and a carboxyhemoglobin determination before autopsy. At necropsy attention will be focused on the appearance and handling of the lungs for proper preservation of this organ for routine staining and/or histochemistry. When the lungs have been properly manipulated, a qualitative description of the appearance of liver and kidney will be made. In addition the adrenals, liver, kidney and heart will be weighed on a tissue torsion balance and the organ weights expressed as percent of body weight.

The details as to lung preparations are as follows: the mouse will be anesthetized by intra-peritoneal injection with 0.2 ml of Sedasol, the trachea exposed and a pair of straight 4-inch iris forceps carefully pushed under the trachea and allowed to open approximately 1.0 cm in order to raise and slightly stretch the trachea to facilitate further manipulations. A 20-gauge 1/2-inch hypodermic needle, snapped off to a 1/4-inch length and fitted with 18 inches of polyethylene tubing (i.d. 0.034) cut to a slight angle at the free end, is attached to a filled 1 ml Luer Loc tuberculin syringe containing diluted O.C.T. compound or histological fixative and all air expressed from the tubing and syringe. A silk suture size 00 is drawn under the midline of the trachea and tied loosely on top with a

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~~Budgetary Statement~~ (For a six month study)

## A. Salaries (Personnel by names)

## Professional

Project Manager (H. Rosenkrantz, Ph.D.) Biochemist

% time

35

Analytical Chemist (M. Hagopian, Ph.D.)

25

Gross Pathologist, Histochemist (J. R. Baker)

35

Immunologist (H. J. Esber, Ph.D.)

2

Amount

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## Technical

## Chemists

150

5,600

Histology and Immunology

35

900

Biology (for smoking and autopsy)

400

10,000

Animal Care

120

2,838

Data Handling

10

350

Sub-Total

27,278

## B. Consumable Supplies (list by categories)

1700 ICR Mice @ 33¢

\$595.00

Rodent Feed and Bedding

220.00

Cigarettes (approx. 60,000

600.00

@ \$10 per thousand

Laboratory supplies (chemicals, glassware, columns, detectors, reagents and disposable items)

2,970.00

Sub-Total

4,385

31,663

## C. Other Expenses (itemize)

Technical Reports

\$200.00

Overtime Premium

100.00

300

Sub-Total

31,963

## D. Permanent Equipment (itemize)

None

## E. Overhead (100% of A)

27,278

Sub-Total

59,241

## F. G&amp;A (12% of above)

7,109

Sub-Total

66,350

## G. Fee (8%)

5,308

Estimated Future Requirements:

Total

\$71,658

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature

Director of Project

Signature

Business Officer of the Institution

Telephone

Telephone

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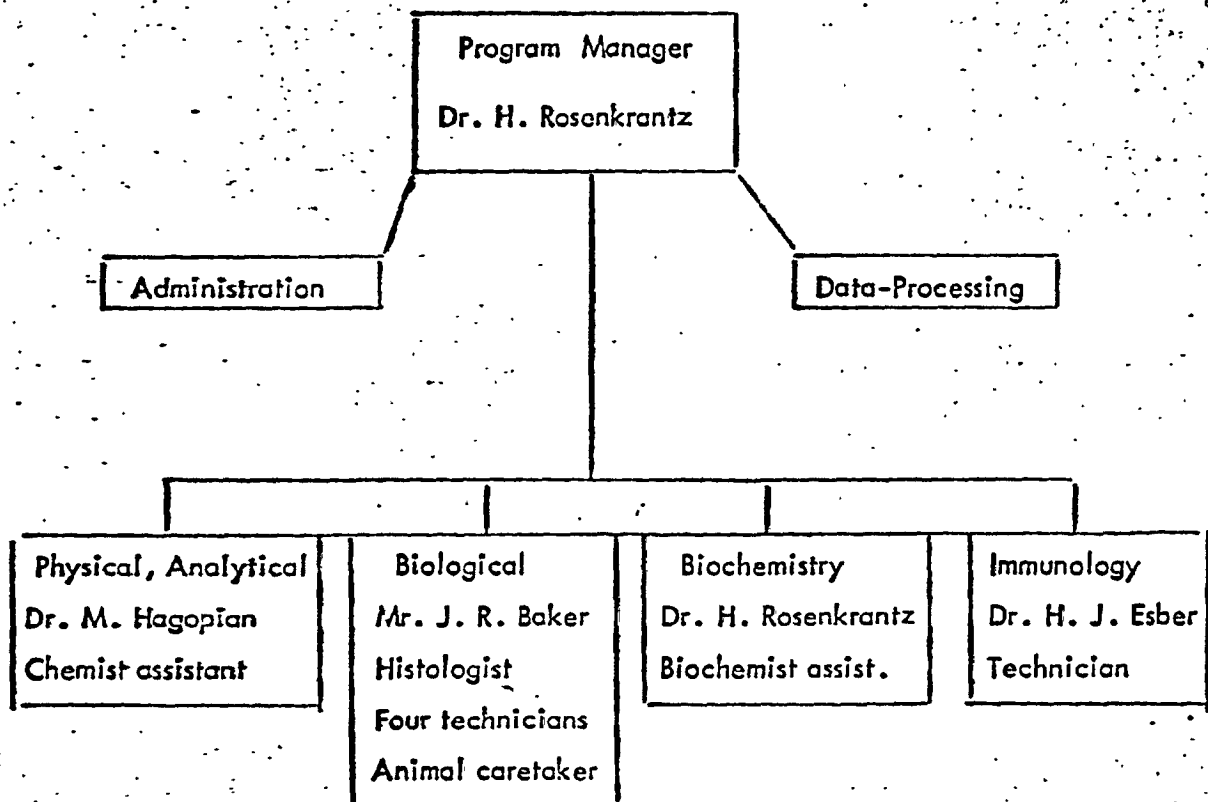
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ANTICIPATED ANIMAL REQUIREMENTS AND UTILIZATION FOR THIS PROJECT

Machine	Smoke Phase	Range Finding Animals*	LD <sub>50</sub>				Continuous				
			Dose	Animals*	Histo-path.	Bio-chem.	Dur.	Animals*	Histo-path.	Bio-chem.	Immun. supp.
1	Whole	20	1 Lo	12	(2)	(2)	7	10			(10)
			2	12	(2)	(2)	14	15	(5)	(10)	
			3	12	(2)	(2)	28	15	(5)	(10)	
			4	12	—	—	56	15	(5)	(10)	
			5 Hi	12	—	—	56 + 14	15	(5)	(10)	
	Gaseous	20	1 Lo	12	(2)	(2)	7	10			(10)
			2	12	(2)	(2)	14	15	(5)	(10)	
			3	12	(2)	(2)	28	15	(5)	(10)	
			4	12	—	—	56	15	(5)	(10)	
			5 Hi	12	—	—	56 + 14	15	(5)	(10)	
	Total		40	120				140			
	2	Total	40	120				140			
	3	Total	40	120				140			
	4	Total	40	120				140			
Sham			60				160				
Cage			60				160				
Totals			160	600				880			
Grand Total				1640 plus							

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VII PROJECT MANAGEMENT

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## VI FACILITIES

The Mason Research Institute now operates nine buildings in the Harvard Street complex. Six are in current use for MRI projects and each has generally been designed for a specific discipline (e.g. immunology, pharmacology, biochemistry, pathology, etc.). The total floor space now used is 45,000 sq. ft. of which one-sixth is for non-technical use.

Each discipline also houses their own small laboratory animals maintaining independent monitoring of the colonies. The advantage of having experimental animals close-by specific laboratories is obvious.

One building is completely devoted to large animals, monkeys, dogs and cats, which contains quarantine areas for holding animals before they are accepted into established ward groups. This building is also outfitted with an extensive treatment room, autopsy room and an experimental surgery.

In all buildings both caging and laboratory equipment is mobile and can be shifted to suit the needs of particular projects. Suitable temperature, humidity and air change systems exist.

At the moment our in-house animal colonies include approximately 130 dogs, 120 monkeys, 200 rabbits, 20 cats, 12,000 rats and 2,000 mice.

### A. Smoking Area

Presently two large rooms are employed for the smoking machines and animal housing. Mice are kept in a room approximately 1600 sq. ft. in size which is directly opposite the smoking area of similar dimensions. Space is available for four smoking machines of the types previously studied.

### B. Gross Pathology, Histochemistry

Histology occupies approximately 810 sq. ft. while 432 sq. ft. are devoted to histochemistry. A photography dark room is also in use. Instrumentation for histology and histochemistry include three automatic tissue processors (Lipshaw

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and Fisher), several microtomes and an American Optical automatic knife sharpener, a freezing microtome (AO Histofreeze), and Ames Lab-Tek microtome cryostat, wax ovens, analytical and tissue balances, slide dryer, Lipshaw-cryoplates, incubator and several research grade microscopes for routine and photomicrography studies. Histology also has a Porter-Blum ultramicrotome that is used for electron microscopy.

#### C. Biochemistry, Analytical Chemistry

Clinical chemistry occupies approximately 1050 sq. ft., biochemistry, 1400 sq. ft. and analytical chemistry, 920 sq. ft. Ancillary laboratories contain facilities for radioisotope experiments, low-temperature tissue fractionation and physico-chemical instrumentation. Equipment includes Beckman and Spectronic 505 spectrophotometers, Turner Fluorometer, pH meters, incubators, Bendix polarimeter, Coleman flame photometers, Spinco and Lourdes ultracentrifuges, Fisher-Johns melting point apparatus, Mettler analytical and tissue balances, column, paper, thin-layer and Aerograph gas chromatography equipment, Loenco gas chromatograph for carbon monoxide determination, Nuclear Chicago scintillation and Geiger-Muller counters and Aminco Warburg apparatuses.

#### D. Immunology

Immunology and serology laboratories occupy approximately 510 sq. ft. and adequate space is available to perform the immunosuppression test on smoked mice. Major instrumentation includes a Branson sonifier, Wiley mill, various Virtis homogenizers, ambient and refrigerated centrifuges, Spinco ultracentrifuge, Cook microtiter apparatus, Gelman immunodiffusion and electrophoresis instrumentation, Virtis lyophilizer, Densicord, and assorted incubators, water baths and autoclaves.

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corresponding groups of mice (8 - 10 smoked, 4 sham-smoked and 4 cage-controls) will be exsanguinated suborbitally at 14, 28, 56 and 70 days and carboxyhemoglobin determined on half the animals. Three pools of sera within each respective group will be used for estimating blood sugar by an adaptation of the anthrone procedure, SGOT and SGPT by the 2,4-dinitrophenylhydrazine method and alkaline phosphatase by the phenyl phosphate procedure.

The exsanguinated animals will be opened, the appearance of the lungs, liver and kidney noted and organ weights of adrenals, liver, lungs, kidney and heart taken. An approximately 100-mg sample of liver and lung, respectively, will be homogenized in distilled water and macromolecules precipitated with cold 10% trichloroacetic acid. The supernatant will be employed for the estimation of lactate by the copper-p-phenylphenol method while the residue is extracted sequentially with alcohol, chloroform and ether to purify the nucleic acids. DNA is determined by the diphenylamine color reaction.

Other samples of lung (ca 50 mg) will be transferred to tared cups and dried to constant weight in an electric oven. Remaining portions of lung and liver, respectively, will be digested in 1-3 ml of 30% potassium hydroxide for 15-20 minutes in a boiling water bath. Subsequently the cooled digests will be diluted to 20-50 ml with distilled water and 0.1 ml used for glycogen determination by the anthrone reaction (20). The number of smoked mice required for the biochemical studies would be 10 mice x 4 periods x 4 machines x 2 smoke phases or 320. An additional 160 will serve as controls.

##### 5. Immunology

An immunological parameter, antibody response, will permit evaluation of each smoking machine on immunosuppression. Separate groups of mice distinct from those used for pathology and biochemistry will be

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Thirdly, the blood from separate groups of animals challenged with sheep red blood cells will be employed for an immunosuppressive test.

## B. Methods of Procedure

### 1. Mechanical evaluation:

Observations will be recorded as to the voltage/cycle requirements of each machine, the method of smoke generation (pressure or suction), the presence and type of a device for varying puff volume and its reproducibility, whether the cigarette is horizontal or vertical or at a particular angle, the ease, stability and time required to exchange cigarettes, the burning time of cigarettes smoked to a 20-mm butt length, ability to control air:smoke ratios, an estimate of directness and distance of the smoke pathway, the time cycle and efficiency of a purge system and the presence of an adapter and the ease of insertion and replacement of Cambridge filters.

Additional observations and measurements will include the animal capacity of each smoking apparatus, the mathematically calculated or water displacement volume of the smoke chamber and/or animal containers, the total animal loading time, the measured distance between butt and animal, the region and percent of body surface exposed, the ability and frequency of mice to block smoke orifices and the capability of visual inspection of animals during smoking cycles.

In relationship to the reference cigarette three factors will be evaluated: 1) puff characteristics, 2) chemical profile of the smoke and 3) feasibility of monitoring carbon monoxide in the smoke chamber. Puff characteristics will be determined by ascertaining if each machine can be adjusted to a 35 ml puff volume of two seconds duration with a 58 second interval between puffs. With such settings, the smoke delivery syringe or flow meter will be read several times for several cigarettes to measure variability of puff volume and number of puffs per cigarette. Several determinations at different times of the day and different days will be performed to estimate puff frequency, interval between puffs, interval

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Dr. Miasnig Hagopian, Analytico-organic Chemist

EDUCATION:

- B. S. Agricultural Chemistry. University of Rhode Island, Kingston, Rhode Island, 1950.  
M. A. Organic Chemistry. Clark University, Worcester, Massachusetts, 1955.  
Ph. D. Organic Chemistry. Clark University, Worcester, Massachusetts, 1965.

PROFESSIONAL EXPERIENCE:

- 1967 - present Biochemist, Mason Research Institute, Worcester, Massachusetts.  
1961 - 1967 Research Biochemist, The Memorial Hospital Research Laboratory, Worcester, Massachusetts.  
1951 - 1961 Research Biochemist, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts.

SOCIETY MEMBERSHIPS:

American Chemical Society  
Sigma XI  
American Association for the Advancement of Science

Dr. Henry J. Esber, Immunologist

EDUCATION:

- B. S. College of William and Mary, Norfolk, Virginia, 1961.  
M. S. University of North Carolina, Chapel Hill, North Carolina, 1963.  
P. H. 1963  
Ph. D. West Virginia University, Morgantown, West Virginia, 1967.

PROFESSIONAL EXPERIENCE:

- 1967 - present Immunologist, Mason Research Institute, Worcester, Massachusetts.  
1967. Postdoctorate, Department of Public Health and Los Angeles County Hospital, Los Angeles, California.  
1964 - 1967 Graduate Research Fellow, West Virginia University, Morgantown, West Virginia.  
1963 - 1964 Bacteriologist-in-charge, Portsmouth General Hospital, Portsmouth, Virginia.  
1958 - 1962 Medical Technician, Leigh Memorial Hospital, Norfolk, Virginia.

SOCIETY MEMBERSHIPS:

American Society for Microbiology  
American Public Health Association

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**EDUCATION:**

Mr. John R. Baker, Histochemist

**Certification:**Medical Technology, Medical Laboratory Techniques, Institute  
Medical Laboratory Technology, London, England, 1943.**Diploma:**Pathology Techniques, Institute Medical Laboratory Technology,  
London, England, 1945.**PROFESSIONAL EXPERIENCE:**

- 1967 - present Head of Histology and Histochemistry, Mason Research Institute  
Worcester, Massachusetts.
- 1959 - 1967 Research Associate, Histochemistry and Pathology, Bio-Research  
Institute and Bio-Research Consultants, Inc., Cambridge,  
Massachusetts.
- 1957 - 1959 Director of Laboratories and Research Assistant, Pathology and  
Research, St. Margaret's Hospital, Dorchester, Massachusetts.
- 1952 - 1957 Research Assistant, Experimental Pathology, Cancer Research  
Unit of Tufts University School of Medicine, Boston, Mass.
- 1948 - 1952 Chief of University Laboratory Staff and Research Assistant,  
Pathology, University College, West Indies.
- 1939 - 1948 Administrator and Chief of Laboratory Staff Pathology and  
Instructor of Histopathological Technique, Post Graduate Medical  
School, London, England.
- 1935 - 1939 Senior Technician, Pathology, Post Graduate Medical School,  
London, England.
- 1933 - 1935 Senior Technician, Central Histological Laboratories, Archway  
Hospital, London, England.
- 1930 - 1933 Research Technician, Charing Cross Hospital Institute of  
Pathology, London, England.
- 1928 - 1930 Technician, Pathology, Radiography, Cardiography, National  
Hospital Diseases of the Heart, London, England

(Illustrations of Baker's human lung preparations are contained  
in the (British) Medical Research Council's Special Report,  
Series No. 250, Chronic pulmonary disease in South Wales  
coal miners).

**SOCIETY MEMBERSHIP:**

Sigma Xi  
Royal Microscopic Society of London  
Royal Society of Health - London  
Society of Medical Technologists - West Indies  
Histochemical Society of America

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It is also understood that there will exist an official monitor liaison between the parties as indicated and appointed by C.T.R.-U.S.A. The monitor must be consulted for questions of procedure and logistics before any changes can be honored. The monitor liaison will be empowered to work with M.R.I., E.G.G. as it pertains to this proposed evaluation.

#### A. Specific Aims of Present Proposal

It is intended to evaluate four smoking machines to be provided by the Council for Tobacco Research, U.S.A. The study will be divided into three phases, 1) mechanical comparison of the machines, 2) establishment of an LD<sub>50</sub> for mice exposed to whole smoke and gaseous phase, respectively, for each machine, and 3) measurement of selected biological parameters during continuous smoking for 56 days. The latter phase will involve sacrifice of animals at sequential intervals of exposure with some animals followed through a recovery period after 56 days of exposure. Separate groups of mice will be employed for whole smoke and gaseous phase treatments. The standard reference cigarette developed by the University of Kentucky will be used throughout.

Phase 1: The four smoking machines will be evaluated as to mechanical specifications, operational characteristics, puff properties and parameters related to animal exposure to smoke. It is anticipated that <sup>adequate</sup> detailed plans, <sup>operating information</sup> diagrams and dimensions will be provided with each smoking apparatus, to facilitate checks on electric power requirements, functions and types of solenoids, microswitches, relays, timers, method of smoke generation, device for varying puff volume, suitability of filtration adapter, purging arrangement, capability of smoke dilution and mouse capacity.

Operational characteristics will be inspected for type and reliability of cigarette ignition, stability of cigarette and cigarette holder geometry, cigarette orientation, cigarette burning time, smoke pathway and cigarette replacement time.

*Rec. operational manual.*

*We will check this.*

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## V BIOGRAPHICAL SKETCHES

Dr. Harris Rosenkrantz, Director of Biochemistry

### EDUCATION:

- A. B. Biology - Chemistry. Brooklyn College, Brooklyn, New York, 1943
- M. S. Biology. New York University, N. Y., 1946.
- M. S. Physiology. Cornell University Medical School, New York, 1948.
- Ph. D. Physiology. Tufts Medical College, Boston, Massachusetts, 1952.

### PROFESSIONAL EXPERIENCE:

- 1959 - present Director of Biochemistry, Mason Research Institute, Worcester, Massachusetts
- 1963 - present Professor of Biochemistry, Clark University, Worcester, Massachusetts.
- 1959 - 1962 Associate Professor of Biology, Clark University, Worcester, Massachusetts.
- 1955 - 1958 Special lecturer in Biochemistry, Clark University, Worcester, Massachusetts.
- 1952 - 1959 Staff Scientist, Worcester Foundation for Experimental Biology and Medicine, Shrewsbury, Massachusetts.
- 1951 - 1952 Research Fellowship, Worcester Foundation for Experimental Biology and Medicine, Shrewsbury, Massachusetts.
- 1948 - 1951 Research Associate, Department of Psychiatry, New York Hospital, New York.
- 1946 - 1948 Research Fellowship, Department of Physiology, Cornell University Medical School, New York.
- 1943 - 1946 Technician, Department of Metabolism, New York Hospital, New York.

### SOCIETY MEMBERSHIPS:

- American Chemical Society
- The American Society of Biological Chemists
- American Association for the Advancement of Science, Fellow
- The Endocrine Society
- The New York Academy of Sciences
- Sigma XI
- The Coblentz Society
- The American Institute of Chemists, Fellow

### AWARDS:

- Recipient of the 1956 Admiral Ralph Earle Award of the Worcester Engineering Society

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## SUMMARY

The mechanical and biological evaluation of four smoking machines is proposed. During phase one, the mechanical and electrical features, the puff characteristics and parameters related to animal exposure will be evaluated for each machine and for both whole smoke and gaseous phase.

Also to be obtained will be <sup>some analytical data on the gaseous + particulate</sup> a gas chromatographic profile and the chemical <sup>phases of cigarette smoke, these determinations being dependent</sup> reducing capacity of the smoke (generated from each apparatus) when a reference <sup>in methods + instrument which will be available for the</sup> cigarette is smoked. <sup>purpose at hand.</sup>

In phase two, mice will be exposed to various doses of smoke in order to establish an LD<sub>50</sub> and a non-lethal dose for 56 days of continuous treatment. Timed carboxyhemoglobin measurements and a minimum of preparative histology and histochemistry will be carried out on these animals.

In phase three, separate groups of mice will be exposed to smoke for specific intervals up to 56 days on each machine and a number of biological parameters will be investigated. These parameters will include organ weights, cytochrome C-oxidase determination in various tissues, five serum endpoints, lung dry weight, DNA, glycogen and lactate, liver glycogen and lactate, and an immunosuppressive test. One group of animals will be permitted to recover for two weeks after 56 days of smoke exposure before tissue analyses are performed. Sham-smoked and cage control mice will also be examined for the same parameters.

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It is understood that the results and developments of this contract will be and remain the property of C.T.R. - U.S.A. That all reports of progress or status are to be and remain in confidence between the two parties (C.T.R. - U.S.A. and M.R.I., E.G.G.). No publications or verbal presentations may be written or expressed without specific written permission obtained not less than 60 days in advance of such anticipated disclosures.